DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892
Building 7
Room 100
(301) 496-4205
FAX: (301) 496-8312

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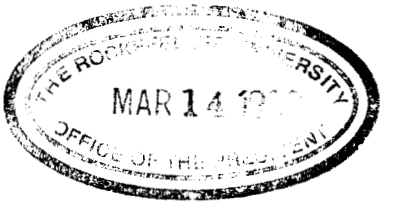


Dr. Joshua Lederberg
President
Rockefeller University
1230 York Avenue
New York, NY 10021

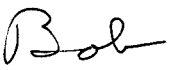
Dear Josh:

Packaging and expression of a foreign gene by influenza A virus was recently achieved by Peter Palese and his colleagues (Cell 57: 1107-1113, 1989). However, the complexity of the procedures employed and the requirement for infection by a helper influenza A virus suggests that this approach does not pose a significant offensive threat at this time. The successful strategy used by Palese's group included: (1) construction of a chimeric cDNA containing the sequence for the coding region of a foreign gene (CAT in this instance) flanked by the 3' and 5' transcriptional, replicative and packaging signals of an influenza A virus gene, (2) transfection of a cRNA transcript of the cDNA in the presence of purified influenza A virus polymerase complex proteins and (3) infection by an influenza A helper virus. It appeared that the chimeric cRNA was amplified and packaged in influenza A virus particles which were not infectious but which could replicate with low efficiency in cells co-infected with a fully competent influenza A helper virus. The low efficiency of replication of the recombinant influenza A virus particles and the requirement for infection with an influenza A helper virus suggests that in vivo infection by recombinant virus particles and transmission to contacts are unlikely to occur.

More ominous are the implications of the most recent study by Palese's group. Previously, it had not been possible to introduce either genetic information derived from cDNA or site-specific mutations into the genome of any negative strand RNA virus. In a manuscript that I have sponsored for publication in PNAS Peter and his associates describe the introduction of an engineered influenza gene, synthesized in vitro from cDNA, into a fully infectious influenza A virus. This means it should be possible to increase the virulence of influenza A virus by introduction of specific mutations at sites known to affect the virulence of avian influenza A viruses. For example, insertion of 3 to 5 basic amino acids (arginine or lysine) immediately upstream of the cleavage site of the hemagglutinin glycoprotein would probably cause a significant increase in virulence of human influenza A viruses. Cleavage of the hemagglutinin by host cell protease(s) is required for

infectivity of influenza A virus and any mutation that increases the efficiency of this cleavage extends cell tropism and increases virulence. Fortunately, none of the H1, H2 or H3 hemagglutinins present on influenza A viruses recovered from humans over the past 57 years (since the recovery of the first human influenza A virus in 1933) possess more than one basic amino acid at the cleavage site. Also, it may be possible to express chimeric genes that encode a foreign protein and an influenza A viral protein. There are several strategies that could be employed to pursue this objective. Each of these prospects is rather frightening.

Sincerely,



Robert M. Chanock, M.D.
Chief
Laboratory of Infectious Diseases
National Institute of Allergy
and Infectious Diseases
National Institutes of Health

cc: Dr. Thomas Monath
Dr. Alexis Shelokov
Dr. John Steinbruner
Ms. Lynn Rusten